Spermatological characters in the Dipylidiidae Stiles, 1896 (Cestoda, Cyclophyllidea)

Jordi Miquel[1]*, Zdzisław Świderski[2,3] and Bernard Marchand[4]

[1]Laboratori de Parasitologia, Departament de Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII, s/n, 08028 Barcelona, Spain; [2]W. Stefański Institute of Parasitology, Polish Academy of Sciences, 51/55 Twarda Street, 00-818 Warsaw, [3]Department of General Biology and Parasitology, Medical University of Warsaw, Poland; [4]Laboratoire Parasites et Écosystèmes Méditerranéens, Faculté des Sciences et Techniques, Université de Corse, 20250 Corte, France

Abstract

The present paper compares the ultrastructural data on spermatology of three dipyliidiid species (*Dipylidium caninum*, *Joyeuxiella echinorhynchoides* and *J. pasqualei*) and establishes a general pattern of spermiogenesis and main ultrastructural characters of the mature spermatozoa for the Dipylidiidae. Spermiogenesis is characterized by an external growth of the flagellum followed by a proximodistal fusion of the latter with the cytoplasmic extension. The centrioles are associated with striated rootlets which are thin in *D. caninum* and well-developed in *Joyeuxiella* spp. The most characteristic features of the mature spermatozoa of the three species are the presence of a single crest-like body and a periaxonemal sheath. Nevertheless, the three compared species differ in the thickness of these structures. Differences are also observed in the length of the apical cone.

Key words

Introduction

The taxonomy of the dilepidid cestodes *sensu lato* at the family level and lower groups has been controversial for a long period of time. Some authors have recognized only one family, Dilepididae Fuhrmann, 1907, with three subfamilies: Dilepidinae Fuhrmann, 1907, Dipylidiinae Stiles, 1896 and Paruterininae Fuhrmann, 1907 (Yamaguti 1959, Schmidt 1986). Other authors have elevated each of these subfamilies to family rank (Matevosyan 1953, 1963; Wardle *et al.* 1974). According to Jones (1994), four families are recognized: Dilepididae Fuhrmann, 1907, Metadilepididae Spasskii, 1959, Paruterinidae Fuhrmann, 1907 and Dipylidiidae Stiles, 1896. The Dipylidiidae corresponds to the subfamily Dipylidiinae of Yamaguti (1959) and Schmidt (1986) and is represented by only three genera, all parasites of carnivores: *Dipylidium* Leuckart, 1863, *Diplopylidium* Beddard, 1913 and *Joyeuxiella* Fuhrmann, 1935 (see Jones 1994).

It has now been clearly demonstrated that ultrastructure of spermiogenesis and the spermatozoon reveal significant characters for phylogenetic inference in parasitic Platyhelminthes (Świderski 1968, 1986; Euzet *et al.* 1981; Justine 1991, 1997,

1998, 2001; Bâ and Marchand 1994a, 1995; Watson and Rohde 1995; Hoberg *et al.* 1997; Świderski and Mackiewicz 2002). In the Cyclophyllidea, data on the ultrastructural details of sperm and spermiogenesis are available for 9 of the 15 families recognized by Khalil *et al.* (1994): Anoplocephalidae Cholodkovsky, 1902, Catenotaeniidae Spasskii, 1950, Davaineidae Braun, 1900, Dilepididae Railliet et Henry, 1909, Dipylidiidae Stiles, 1896, Hymenolepididae Ariola, 1899, Mesocestoididae Fuhrmann, 1907, Nematotaeniidae Lühe, 1910 and Taeniidae Ludwig, 1886.

Ultrastructural studies of the male gametes from the Dipylidiidae have been done on two genera: *Dipylidium* (Miquel and Marchand 1997, Miquel *et al.* 1998) and *Joyeuxiella* (Ndiaye *et al.* 2003a).

According to Jones (1983) the genus *Joyeuxiella* comprises only three valid species: *J. echinorhynchoides* (Sonsino, 1889), *J. pasqualei* (Diamare, 1893) and *J. fuhrmanni* (Baer, 1924).

Two species of the genus *Joyeuxiella*, *J. echinorhynchoides* and *J. pasqualei*, have been analyzed previously in detail (see Ndiaye *et al.* 2003a). Following the latter study, the available data on spermiogenesis and spermatozoon of the genus
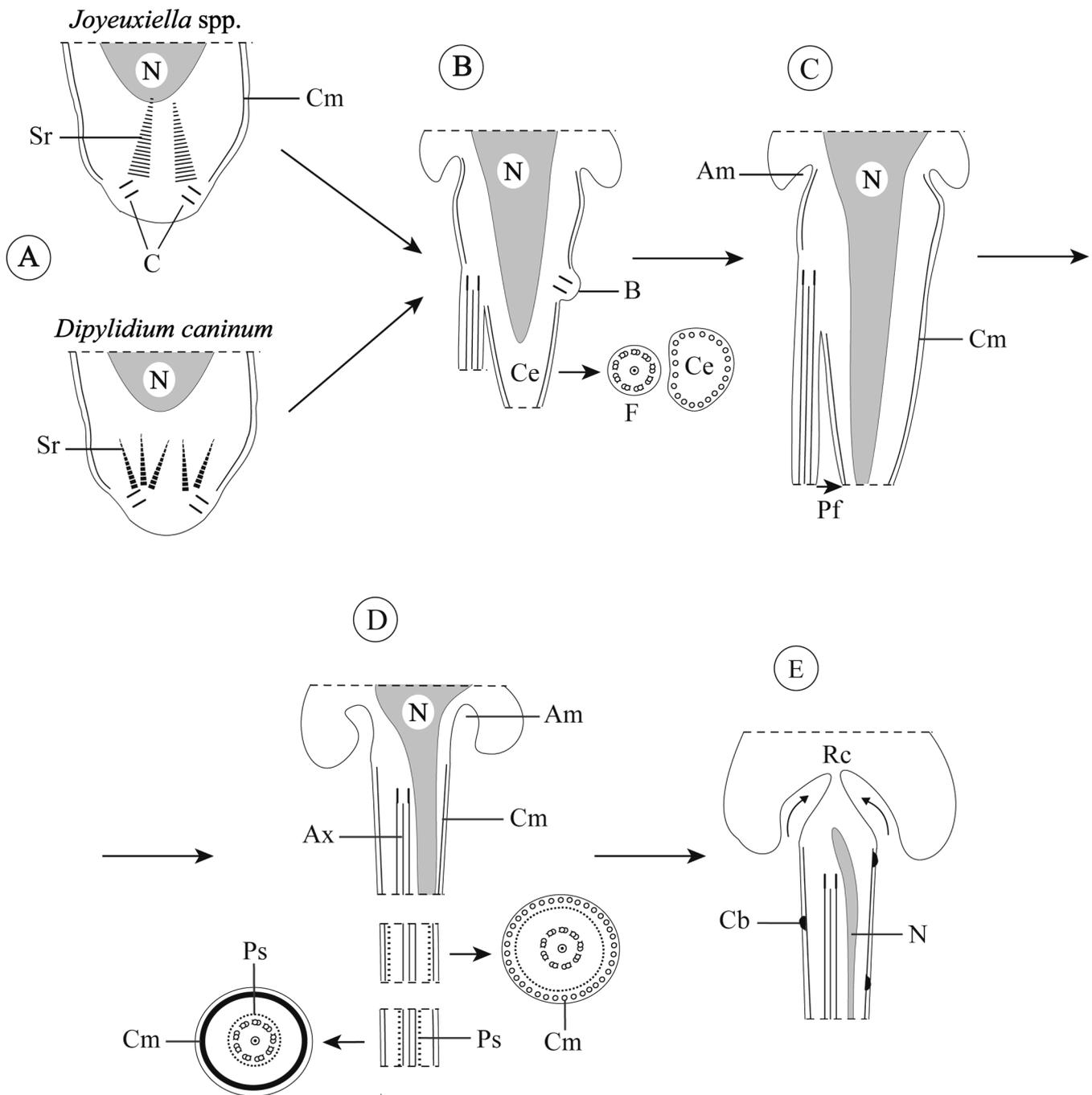
Fig. 1A-E. Diagram showing the main stages of spermiogenesis in Dipylidiidae species. **Abbreviations to all figures:** Ac – apical cone; Am – arching membranes; Ase – anterior spermatozoon extremity; Ax – axoneme; B – cytoplasmic bud; C – centriole; Cb – crest-like body; Ce – cytoplasmic extension; Cm – cortical microtubules; F – free flagellum; G – glycogen-like granules; N – nucleus; Pf – proximodistal fusion; Ps – periaxonemal sheath; Rc – residual cytoplasm; Sr – striated root

Joyeuxiella allow us to consider a definitive status of this genus. In addition, three studies (Miquel and Marchand 1997, Miquel *et al*. 1998, Ndiaye *et al*. 2003a) described the complete list of spermatological characters for three species belonging to two of the three genera of dipylidiids. Presently, for all representatives of Dipylidiidae, only data on the genus *Diplopylidium* are lacking. The purpose of the present comparative study is to establish a general pattern of spermiogenesis and the main ultrastructural characters and organization of the mature spermatozoon in this family of cyclophyllideans.

Materials and methods

Materials

Mature specimens of *Dipylidium caninum* were collected live from the small intestine of dogs (*Canis familiaris*) coming from the Zoonoses Service of Barcelona (Spain). Samples of *Joyeuxiella pasqualei* were collected from the intestinal tract of a road-killed wild cat (*Felis lybica*) from Thiès (Senegal). Finally, the mature proglottids of *Joyeuxiella echinorhynchoides* were collected from the intestine of a road-killed red fox (*Vulpes vulpes*) from Corte (Corsica, France).

Methods

Naturally infected dogs were killed in the Zoonoses Service of Barcelona by means of intramuscular Zoletil® injection (tiletamine hydrochloride and zolazepam hydrochloride) followed by intracardiac Dexeutanol® [5-ethyl-5-(1-methylbutyl) barbituric acid sodium salt] injection. The three species of dipylidiid cestodes: *D. caninum*, *J. echinorhynchoides* and *J. pasqualei*, were placed in a 0.9% NaCl solution. Mature proglottids of these cestodes were routinely processed for transmission electron microscopic (TEM) examination; they were fixed in cold (4°C) 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.2 for 1 h, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.2, postfixed in cold (4°C) 1% osmium tetroxide in the same buffer for 1 h, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.2, dehydrated in an ethanol series and propylene oxide, and finally embedded in Spurr epoxy medium. Ultrathin sections were obtained using a Reichert-Jung Ultracut E ultramicrotome, placed on copper grids and double-stained with uranyl acetate and lead citrate. Ultrathin sections were examined using Hitachi H-600 and Jeol 1010 electron microscopes in the Scientific Services of the University of Barcelona.

Results

Spermiogenesis

Spermiogenesis in *D. caninum*, *J. echinorhynchoides* and *J. pasqualei*, starts with the formation of a differentiation zone. This is a conically shaped area bordered by submembranous cortical microtubules containing two centrioles. Each of these centrioles is associated with striated rootlets and exhibits a cytoplasmic process between them (Figs 1A, 2 and 3). These striated rootlets are reduced in size and are very thin in *D. caninum* (Figs 1A and 2). On the other hand, in both *Joyeuxiella* species striated rootlets are well-developed (Figs 1A and 3). One of the centrioles gives rise to a flagellum that continues its growth parallel to the cytoplasmic expansion (Figs 1B, 2 and 6). The other centriole is situated in a short cytoplasmic bud and subsequently aborts (Fig. 1B). Afterwards, a proximodistal fusion occurs between the axoneme and the cytoplasmic expansion (Fig. 1B, C). Cortical microtubules undergo elongation parallel to the sperm axis and then become twisted (Figs 1D, 4 and 5). A ring of arched membranes is present at the base of the differentiation zone (Figs 1B-D, 2–5). The nucleus elongates, becomes conical and migrates along the spermatid body, before the proximodistal fusion of the flagellum takes place (Fig. 1B, C). In the species of *Joyeuxiella*, after the proximodistal fusion, a condensation of cytoplasmic material at the periphery of the spermatid transforms into a striated peripheral layer. At this stage, cortical microtubules are still parallel to the sperm axis (Figs 1D and 7). Later, this striated structure surrounds the axoneme and cortical microtubules become twisted (Figs 1D and 8). The process of cytoplasmic condensation and posterior twisting of cortical microtubules probably plays an important role in the origin of the periaxonemal sheath present in the mature sperm of the three species. Finally, an electron-dense material appears between the cortical microtubules in the anterior part of the differentiation zone. This electron-dense material progressively fuses and forms a crest-like body that appears at the anterior part of the old spermatid in the final stage of spermiogenesis (Figs 1E and 4). At this stage, the nucleus has not yet finished its migration along the spermatid body. The ring of arched membranes narrows until the differentiated spermatozoid detaches from the residual cytoplasm (Figs 1E and 5).

Spermatozoon

The mature spermatozoa of *D. caninum*, *J. echinorhynchoides* and *J. pasqualei* are long filiform cells which lack mitochondria. They are tapered at both extremities and present an axoneme of the 9 + '1' pattern of the trepaxonematan Platyhelminthes.

The anterior extremity of the spermatozoon has an electron-dense apical cone that is about 600 nm long in *D. caninum* and measures more than 2000 nm in length in *Joyeuxiella* species (Figs 9 and 10). The anterior axonemal extremity is situated immediately below the apical cone. The centriole remains clearly visible in this extremity. The axoneme is surrounded by a thin layer of electron-lucent material, a periaxonemal sheath and slightly electron-dense cytoplasm. Cortical microtubules are twisted at an angle of about 40° with respect to the spermatozoon axis in all the three dipylidiid species. Externally, these spirally coiled cortical microtubules are partially surrounded by a thick helicoidal cord of electron-dense material that forms a single crest-like body (Figs 12–14). This crest-like body is coiled at the same angle with respect to the spermatozoon axis and has a maximal width of 150 nm for *D. caninum* and *J. echinorhynchoides* and 75 nm for *J. pasqualei*. The thickness of the crest-like body gradually decreases toward its end.

An intermediate portion anterior the nuclear region is characterized by the absence of a helicoidal crest-like body and by the presence of a periaxonemal sheath that encircles the axoneme (Figs 11, 15–17). In the case of *J. echinorhynchoides* and *J. pasqualei*, this prenuclear region is also characterized by the presence of glycogen-like granules arranged as two loose spiral cords that appear on two opposite sides in
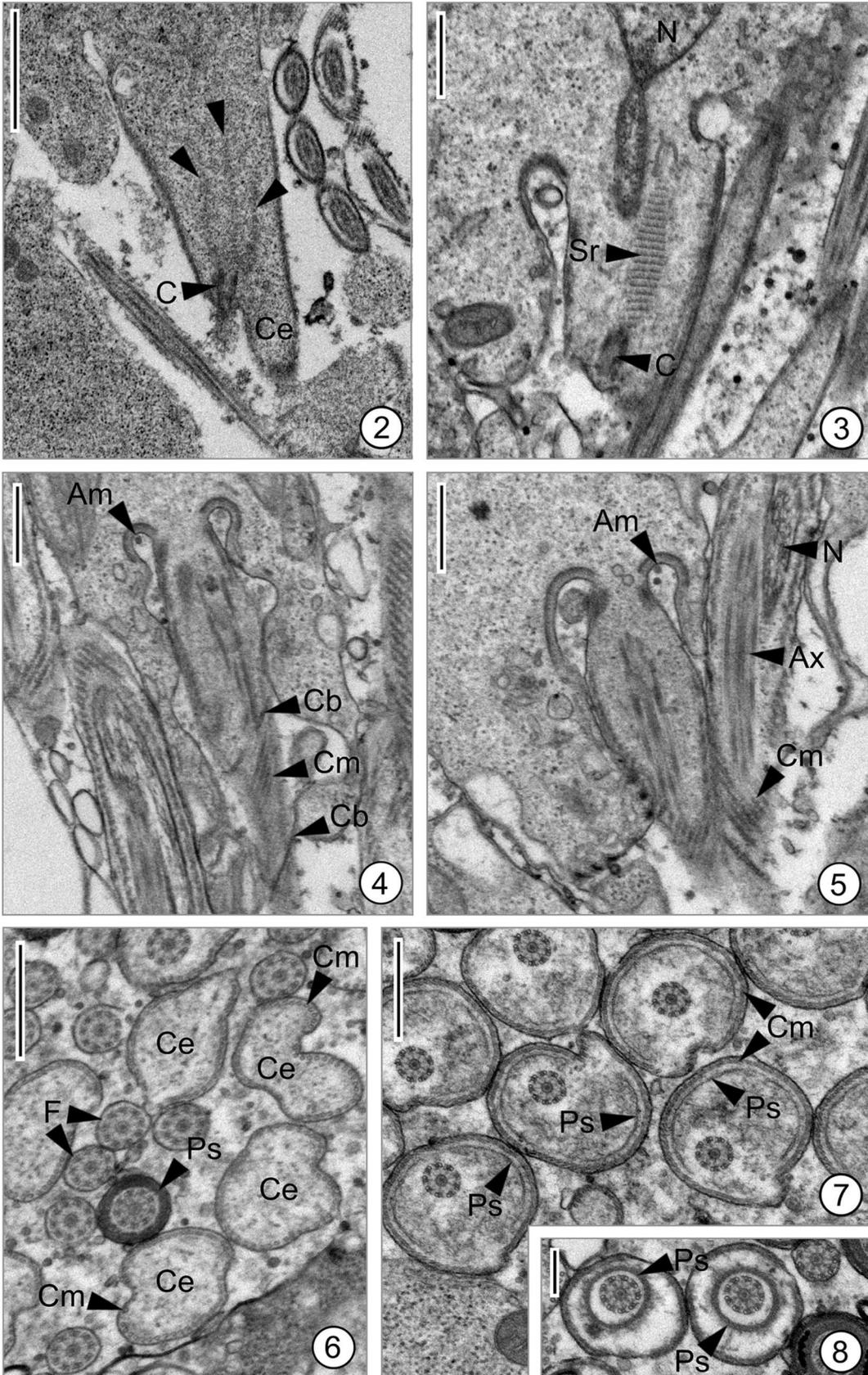
the cross-sections (Figs 11, 15 and 16). These granules are between the periaxonemal sheath and the submembranous layer of cortical microtubules.

More posteriorly, the nucleus appears and coils around the axoneme in a helicoidal form; it occupies the sheath position (Figs 15 and 18). In cross-sections, it appears horseshoe-shaped or almost annular in form.

Finally, the posterior region lacks a nucleus and periaxonemal sheath. The cortical microtubules form a spiralled submembranous layer in the initial part of this region. In *Joyeuxiella* spp., the cortical microtubules become parallel to the spermatozoon axis and progressively disappear. Only the axoneme is observed in more distal areas of this region, and its doublets are progressively disorganized into singlets. A few axonemal microtubules extend to the posterior extremity of the spermatozoon. In *D. caninum*, the axoneme progressively disappears before the end of the cortical microtubules. The posterior tip of the mature sperm of *D. caninum* still shows the presence of twisted cortical microtubules.

Discussion

Spermiogenesis

The most interesting feature of dipylidiid spermiogenesis is the presence of striated rootlets in the differentiation zone and associated with the centrioles. These rootlets can be considered a plesiomorphic character for this cyclophyllidean family. In fact, striated rootlets are generally present during spermiogenesis of the lower cestodes. To date, striated rootlets have been observed in the differentiation zone of the caryophyllidean *Glaridacris catostomi* (Świderski and Mackiewicz 2002), the pseudophyllideans *Bothriocephalus clavibothrium* (Świderski and Mokhtar-Maamouri 1980) and *Eubothrium crassum* (Bruňanská *et al.* 2001), the haplobothriidean *Haplobothrium globuliforme* (MacKinnon and Burt 1985), the trypanorhynchid *Lacistorhynchus tenuis* (Euzet *et al.* 1981, Świderski 1994), the onchobothriids and phyllobothriids of tetraphyllideans *Acanthobothrium benedeni* (Mokhtar-Maamouri and Świderski 1975), *Acanthobothrium filicolle* (Mokhtar-Maamouri 1982), *Onchobothrium uncinatum* (Mokhtar-Maamouri and Świderski 1975), *Phyllo-*

bothrium gracile (Mokhtar-Maamouri 1979), *Phyllobothrium lactuca* (Sène *et al.* 1999) and *Trilocularia acanthiaevulgaris* (Mahendrasingam *et al.* 1989), the proteocephalideans *Proteocephalus longicollis* (Świderski 1985, Bruňanská *et al.* 2004), *Proteocephalus torulosus* (Bruňanská *et al.* 2003) and *Nomimoscolex* sp. (Sène *et al.* 1997), and the tetrabothriidean *Tetrabothrius erostris* (Stoitsova *et al.* 1995). In the Cyclophyllidea, these structures have been described in the three dipylidiid species which are compared in the present study (*D. caninum*, *J. echinorhynchoides* and *J. pasqualei*: see Miquel *et al.* 1998, Ndiaye *et al.* 2003a). In addition, striated rootlets have been reported in the anoplocephalid *Anoplocephaloides dentata* (Miquel and Marchand 1998); and the mesocestoidid *Mesocestoides litteratus* (Miquel *et al.* 1999). In *A. dentata* (Miquel and Marchand 1998) the striated rootlets are thin and quite similar to those in *D. caninum* (Miquel *et al.* 1998). On the other hand, the striated rootlets associated with the centrioles are well-developed in *M. litteratus* (Miquel *et al.* 1999) as well as in the genus *Joyeuxiella* (Ndiaye *et al.* 2003a).

The case of *M. litteratus* is particularly interesting considering that during spermiogenesis, in addition to striated rootlets, a flagellar rotation of 90° and a reduced intercentriolar body were also observed. These characters represent the plesiomorphic conditions within the Eucestoda (Bâ and Marchand 1995; Justine 1998, 2001). Also in *M. litteratus*, the parallel arrangement of the cortical microtubules in the male gamete, another plesiomorphic character, is not in accordance with the general pattern found in the cyclophyllideans (Miquel *et al.* 1999, Justine 2001). Moreover, the life cycle of *Mesocestoides* in which three hosts are required, as well as the morphoanatomy of the genital atrium and the bilobated vitelline gland of representatives of this family (Mesocestoididae) has lead to much controversy regarding the position of this group within the Cyclophyllidea (Wardle *et al.* 1974, Brooks *et al.* 1991, Rausch 1994). Mariaux (1998) suggested that on the basis of molecular data the Mesocestoididae should be removed from the Cyclophyllidea. According to this author, the monophyly of the cyclophyllideans is recognized only when species of the Mesocestoididae are excluded from the Cyclophyllidea or when the representatives of the Tetrabothriidea are reincluded as a family in the Cyclophyllidea. Ultrastructural observations on the spermiogenesis of *M. lit-*

Fig. 2. Three thin striated rootlets (arrow heads) associated with a centriole, giving rise to externally growing flagellum, separated from cytoplasmic extension on the longitudinal section to the differentiation zone of *D. caninum*. Scale bar = 1 µm. **Fig. 3.** Longitudinal section of the zone of differentiation of *J. echinorhynchoides* showing a well-developed striated rootlet associated with a centriole. Note that the migrating nucleus and the centriole are situated in the common cytoplasmic extension. Scale bar = 0.5 µm. **Fig. 4.** Oblique section of the differentiation zone of *J. echinorhynchoides*. Note: (1) the formation of crest-like body in the advanced stage of spermiogenesis, (2) several cortical microtubules situated between the spiral of crest-like body, and (3) arching membrane bound clefts at the base of differentiation zone. Scale bar = 0.5 µm. **Fig. 5.** Two differentiating spermatids of *J. echinorhynchoides* on the oblique sections through the different regions. Note: (1) the region adjacent to arching membrane bound clefts showing a ring of moderately electron-dense material and the obliquely section of axoneme; and (2) the more distal region containing nucleus and the axoneme in the same cytoplasmic extension. Scale bar = 0.5 µm. **Fig. 6.** *Joyeuxiella echinorhynchoides*: cross-sections of a single spermatozoon with axoneme surrounded by a periaxonemal sheath as well as numerous free flagella and cytoplasmic extensions before the proximodistal fusion of both structures. Scale bar = 0.5 µm. **Figs 7 and 8.** Cross-sections of advanced stages of spermatid differentiation in *J. echinorhynchoides* showing early (Fig. 7) and more advanced (Fig. 8) phase of periaxonemal sheath formation. Scale bars for Fig. 7 = 0.5 µm; and for Fig. 8 = 0.2 µm
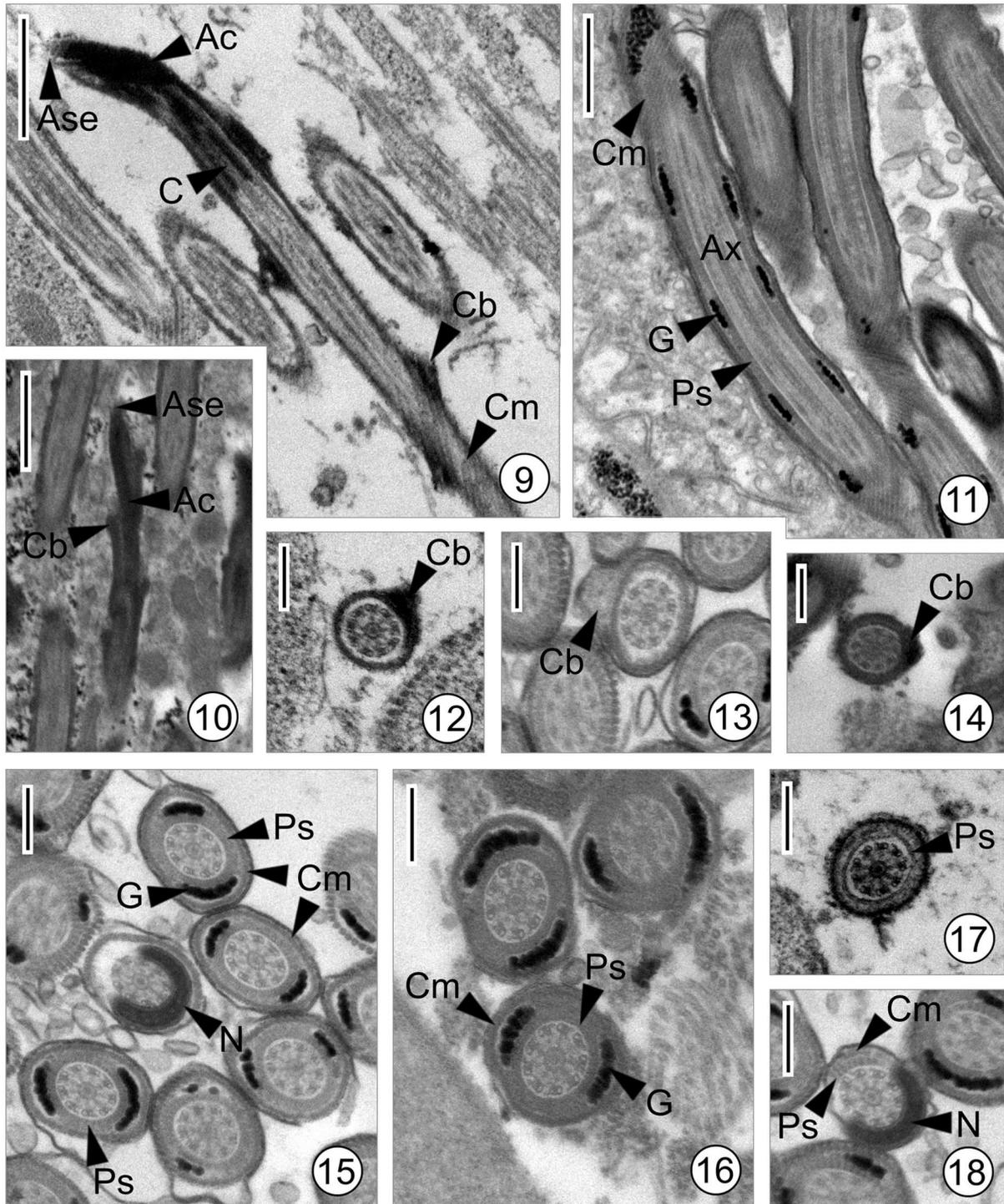
Figs 9 and **10.** The anterior spermatozoon extremity with an apical cone and a single twisted crest-like body on the longitudinal sections of mature spermatozoa of *D. caninum* (Fig. 9) and *J. pasqualei* (Fig. 10). Scale bars = 0.5 μm. **Fig. 11.** Two cords of glycogen-like granules in loose spirals at the opposite parts of the section between the cortical microtubules and the periaxonemal sheath on the longitudinal section of mature sperm of *J. echinorhynchoides*. Scale bar = 0.5 μm. **Figs 12–14.** Details of crest-like body on the cross-sections of anterior region of the mature spermatozoa of *D. caninum* (Fig. 12), *J. echinorhynchoides* (Fig. 13) and *J. pasqualei* (Fig. 14). Scale bars = 0.2 μm. **Figs 15** and **18.** Several cross-sections of mature spermatozoa of *J. echinorhynchoides* showing crescent shape of nucleus and the dense glycogen-like granules situated between the cortical microtubules and the periaxonemal sheath at the opposite poles of the sections (Fig. 15). Note presence of the fragmented periaxonemal sheath in the nuclear region before its complete disappearance (Fig. 18). Scale bars = 0.2 μm. **Figs 16** and **17.** Details showing different thickness of periaxonemal sheath on the cross-sections of the spermatozoa of *J. pasqualei* (Fig. 16) and *D. caninum* (Fig. 17). Scale bars = 0.2 μm

teratus (Miquel *et al*. 1999) are in agreement with this suggestion. Also, the sperm ultrastructure of *M. litteratus* reveals new discriminating characters that also support this exclusion and its placement as a sister group to the tetrabothriideans (see Miquel *et al*. 1999, Justine 2001). All these observations place the validity of the current systematic status of mesocestoidids in question. In what refers to dipylidiids, the presence of typical striated rootlets in *Joyeuxiella* spp. probably should be considered either as a secondary loss or reversal of this synapomorphy in the genus *Joyeuxiella*. In this sense, as previously established for the intercentriolar body (see Justine 2001), it is probably necessary to consider this feature as a multistate character, taking the following conditions into account: (1) the absence of striated rootlets, (2) the presence of atypical striated rootlets, and (3) the presence of typical striated rootlets.

Spermatozoon

The mature spermatozoa of the three dipylidiid species compared in this study are characterized by having both a single crest-like body in the anterior extremity and a periaxonemal sheath (Miquel and Marchand 1997, Ndiaye *et al*. 2003a). Other important details, such as: (1) thickness of the crest-like body; (2) length of the apical cone; (3) thickness of the periaxonemal sheath; and (4) the morphology of the posterior extremity of the spermatozoon, represent distinctive features between the two genera of the Dipylidiidae examined in this study (see Table I).

To date, the ultrastructural studies of the spermatozoon of the Cyclophyllidea reveal that a periaxonemal sheath is present in only 17 species from six families: *Inermicapsifer guineensis* and *I. madagascariensis* (Bâ and Marchand 1994b), *Mathevotaenia herpestis* (Bâ and Marchand 1994c), and *Stilesia globipunctata* (Bâ and Marchand 1992) (Anoplocephalidae), *Catenotaenia pusilla* (Hidalgo *et al*. 2000), and *Skrjabinotaenia lobata* (Miquel *et al*. 1997) (Catenotaeniidae), *Cotugnia polyacantha* (Bâ and Marchand 1994a), and *Raillietina tunetensis* (Bâ and Marchand 1994d) (Davaineidae), *Dilepis undula* (Świderski *et al*. 2000) (Dilepididae), *Echinococcus multilocularis* (Barret and Smyth 1983, Shi *et al*.

1994), *Taenia* spp. (Tian *et al*. 1998), *T. hydatigena* (Featherston 1971), *T. mustelae* (Miquel *et al*. 2000), *T. parva* (Ndiaye *et al*. 2003b), *T. solium* (Willms *et al*. 2003), *T. crassiceps* (Willms *et al*. 2004) (Taeniidae) and the three Dipylidiidae compared here (*D. caninum*, *J. echinorhynchoides* and *J. pasqualei*) (Miquel and Marchand 1997, Ndiaye *et al*. 2003a). According to Ndiaye *et al*. (2003a) the formation of the periaxonemal sheath occurs during the final stages of spermiogenesis; its formation was described for the first time in *Joyeuxiella* spp. The process begins as a condensation of cytoplasm into an electron-dense material that forms a striated structure that encircles the axoneme at the periphery of the spermatid. At this stage the cortical microtubules are still parallel to the presumed spermatozoon axis. However, in more advanced stages of sperm development, the striated structure progressively approaches the axoneme and forms the periaxonemal sheath. It is interesting to note that in this stage of spermiogenesis, the cortical microtubules become twisted. This twisting of cortical microtubules probably results in the displacement and final disposition of the periaxonemal sheath very close to the axoneme.

A comparative analysis of the mature spermatozoon of *J. echinorhynchoides* and *J. pasqualei* has revealed the same ultrastructural organization in both species (Ndiaye *et al*. 2003a). Minor differences were noted only in the maximal width of the single crest-like body (Table I). In *J. echinorhynchoides* it is twice as large as in *J. pasqualei* (150 vs. 75 nm).

The ultrastructural features of the mature spermatozoa of *J. echinorhynchoides* and *J. pasqualei* (Ndiaye *et al*. 2003a) are quite different from those of *D. caninum* (Miquel and Marchand 1997). The most remarkable differences are: (1) at the level of the apical cone which is shorter in *Dipylidium* species (600 nm) and longer in *Joyeuxiella* species (2000 nm); (2) thickness of the periaxonemal sheath in the three species examined; (3) presence of glycogen in *Joyeuxiella* spp. and its absence in *D. caninum*; and (4) the morphology of the posterior extremity of the sperm (see Table I). On the other hand, a periaxonemal sheath seems to be a character present in the spermatozoa of the Dipylidiidae (see above).

Table I. Some differences in spermiogenesis and ultrastructure of the spermatozoon in the dipylidiid cyclophyllideans

Spermatological character	*Dipylidium caninum* Miquel and Marchand (1997) Miquel *et al*. (1998) Present study	*Joyeuxiella pasqualei* Ndiaye *et al*. (2003a) Present study	*J. echinorhynchoides* Ndiaye *et al*. (2003a) Present study
Striated rootlets	thin	well-developed	well-developed
Thickness of crest-like body (nm)	150	75	150
Length of apical cone (nm)	600	>2000	>2000
Thickness of periaxonemal sheath (nm)	15	50–75	40–60
Cortical microtubules in posterior extremity	twisted	parallel	parallel

The disposition of glycogen in two spiral cords in the mature spermatozoon of *Joyeuxiella* species (Ndiaye *et al.* 2003a) has never been reported previously in other cestodes. According to Świderski (1968) and Euzet *et al.* (1981), glycogen seems to be the energy source of the spermatozoon, but the mechanisms by which it is utilized are not yet known. In the Cyclophyllidea, the distribution of glycogen varies greatly in different species.

The ultrastructure of the posterior end of the spermatozoon in *Joyeuxiella* is different from that observed in *D. caninum*. While in *Joyeuxiella* spp., cross-sections of the posterior end present only singlets resulting from the disorganization of the axonemal doublets (Ndiaye *et al.* 2003a), in *D. caninum* the posterior end is characterized by the presence of torsioned microtubules and a moderately electron-dense granular material (Miquel and Marchand 1997).

We believe that further ultrastructural studies on spermatology are necessary to better understand phylogenetic relationships within the Dipylidiidae. In the future, priority should be given to the genus *Diplopylidium* for which ultrastructural data on sperm and spermiogenesis are not yet available.

References

Bâ C.T., Marchand B. 1992. Ultrastructural particularities of the spermatozoon of *Stilesia globipunctata* (Cestoda) parasite of the small intestine of sheep and goats in Senegal. *Journal of Submicroscopic Cytology and Pathology*, 24, 29–34.

Bâ C.T., Marchand B. 1994a. Similitude ultrastructurale des spermatozoïdes de quelques Cyclophyllidea. *Parasite*, 1, 51–55.

Bâ C.T., Marchand B. 1994b. Comparative ultrastructure of the spermatozoa of *Inermicapsifer guineensis* and *I. madagascariensis* (Cestoda, Anoplocephalidae, Inermicapsiferinae) intestinal parasites of rodents in Senegal. *Canadian Journal of Zoology*, 72, 1633–1638.

Bâ C.T., Marchand B. 1994c. Ultrastructure of spermiogenesis and the spermatozoon of *Mathevotaenia herpestis* (Cestoda), intestinal parasite of *Atelerix albiventris* in Senegal. *Acta Zoologica* (*Stockholm*), 75, 167–175.

Bâ C.T., Marchand B. 1994d. Ultrastructure of spermiogenesis and the spermatozoon of *Raillietina* (*Raillietina*) *tunetensis* (Cyclophyllidea, Davaineidae) intestinal parasite of turtle doves in Senegal. *International Journal for Parasitology*, 24, 237–248.

Bâ C.T., Marchand B. 1995. Spermiogenesis, spermatozoa and phyletic affinities in the Cestoda. In: *Advances in spermatozoal phylogeny and taxonomy* (Eds. B.G.M. Jamieson, J. Ausió and J.-L. Justine). *Mémoires du Muséum National d'Histoire Naturelle, Paris*, 166, 87–95.

Barrett N.J., Smyth J.D. 1983. Observations on the structure and ultrastructure of sperm development in *Echinococcus multilocularis*, both *in vitro* and *in vivo*. *Parasitology*, 87, li.

Brooks D.R., Hoberg E.P., Weekes P.J. 1991. Preliminary phylogenetic systematic analysis of the major lineages of the Eucestoda (Platyhelminthes, Cercomeria). *Proceedings of the Biological Society of Washington*, 104, 651–668.

Bruňanská M., Nebesářová J., Scholz T. 2003. Spermiogenesis in the proteocephalidean cestode *Proteocephalus torulosus* (Batsch, 1786). *Parasitology Research*, 90, 318–324.

Bruňanská M., Nebesářová J., Scholz T., Fagerholm H.P. 2001. Spermiogenesis in the pseudophyllid cestode *Eubothrium crassum* (Bloch, 1779). *Parasitology Research*, 87, 579–588.

Bruňanská M., Scholz T., Nebesářová J. 2004. Reinvestigation of spermiogenesis in the proteocephalidean cestode *Proteocephalus longicollis* (Zeder, 1800). *Journal of Parasitology*, 90, 23–29.

Euzet L., Świderski Z., Mokhtar-Maamouri F. 1981. Ultrastructure comparée du spermatozoïde des cestodes. Relations avec la phylogénèse. *Annales de Parasitologie* (*Paris*), 56, 247–259.

Featherston D.W. 1971. *Taenia hydatigena*. III. Light and electron microscope study of spermatogenesis. *Zeitschrift für Parasitenkunde*, 37, 148–168.

Hidalgo C., Miquel J., Torres J., Marchand B. 2000. Ultrastructural study of spermiogenesis and the spermatozoon in *Catenotaenia pusilla*, an intestinal parasite of *Mus musculus*. *Journal of Helminthology*, 74, 73–81.

Hoberg E.P., Mariaux J., Justine J.-L., Brooks D.R., Weekes P.J. 1997. Phylogeny of the orders of the Eucestoda (Cercomeromorphae) based on comparative morphology: historical perspectives and a new working hypothesis. *Journal of Parasitology*, 83, 1128–1147.

Jones A. 1983. A revision of the cestode genus *Joyeuxiella* Fuhrmann, 1935 (Dilepididae: Dipylidiinae). *Systematic Parasitology*, 5, 203–213.

Jones A. 1994. Family Dipylidiidae Stiles, 1896. In: *Keys to the cestode parasites of vertebrates* (Eds. L.F. Khalil, A. Jones and R.A. Bray). CAB International, Wallingford, 555–558.

Justine J.-L. 1991. Phylogeny of parasitic Platyhelminthes: a critical study of synapomorphies proposed on the basis of the ultrastructure of spermiogenesis and spermatozoa. *Canadian Journal of Zoology*, 69, 1421–1440.

Justine J.-L. 1997. La classification générale des Plathelminthes parasites: changements récents et utilisation des caractères ultrastructuraux, en particulier des spermatozoïdes. *Bulletin de la Société Française de Zoologie*, 122, 226–277.

Justine J.-L. 1998. Spermatozoa as phylogenetic characters for the Eucestoda. *Journal of Parasitology*, 84, 385–408.

Justine J.-L. 2001. Spermatozoa as phylogenetic characters for the Platyhelminthes. In: *Interrelationships of the Platyhelminthes* (Eds. D.T.J. Littlewood and R.A. Bray). Taylor and Francis, London & New York, 231–238.

Khalil L.F., Jones A., Bray R.A. 1994. Keys to the cestode parasites of vertebrates. CAB International, Cambridge.

MacKinnon B.M., Burt M.D.B. 1985. Ultrastructure of spermatogenesis and the mature spermatozoon of *Haplobothrium globuliforme* Cooper, 1914 (Cestoda: Haplobothrioidea). *Canadian Journal of Zoology*, 63, 1478–1487.

Mahendrasingam S., Fairweather I., Halton D.W. 1989. Spermatogenesis and the fine structure of the mature spermatozoon in the free proglottis of *Trilocularia acanthiaevulgaris* (Cestoda, Tetraphyllidea). *Parasitology Research*, 75, 287–298.

Mariaux J. 1998. A molecular phylogeny of the Eucestoda. *Journal of Parasitology*, 84, 114–124.

Matevosyan E.M. 1953. Re-organisation of the classification of dilepidid cestodes. Papers on helminthology presented to Academician K.I. Skryabin on his 75th birthday. Izd. AN SSSR, Moscow, 392–397 (In Russian).

Matevosyan E.M. 1963. Principles of cestodology. Vol. III. Dilepidoidea, tapeworms of domestic and wild animals. AN SSSR, Moscow (In Russian).

Miquel J., Bâ C.T., Marchand B. 1997. Ultrastructure of the spermatozoon of *Skrjabinotaenia lobata* (Cyclophyllidea, Catenotaeniidae), intestinal parasite of *Apodemus sylvaticus* (Rodentia, Muridae). *Journal of Submicroscopic Cytology and Pathology*, 29, 521–526.

Miquel J., Bâ C.T., Marchand B. 1998. Ultrastructure of spermiogenesis of *Dipylidium caninum* (Cestoda, Cyclophyllidea, Dipylidiidae), an intestinal parasite of *Canis familiaris*. *International Journal for Parasitology*, 28, 1453–1458.

Miquel J., Feliu C., Marchand B. 1999. Ultrastructure of spermiogenesis and the spermatozoon of *Mesocestoides litteratus* (Cestoda, Mesocestoididae). *International Journal for Parasitology*, 29, 499–510.

Miquel J., Hidalgo C., Feliu C., Marchand B. 2000. Sperm ultrastructure of *Taenia mustelae* (Cestoda, Taeniidae), an intestinal parasite of the weasel, *Mustela nivalis* (Carnivora). *Invertebrate Reproduction and Development*, 38, 43–51.

Miquel J., Marchand B. 1997. Ultrastructure of the spermatozoon of *Dipylidium caninum* (Cestoda, Cyclophyllidea, Dilepididae), an intestinal parasite of *Canis familiaris*. *Parasitology Research*, 83, 349–355.

Miquel J., Marchand B. 1998. Ultrastructure of spermiogenesis and the spermatozoon of *Anoplocephaloides dentata* (Cestoda, Cyclophyllidea, Anoplocephalidae), an intestinal parasite of Arvicolidae rodents. *Journal of Parasitology*, 84, 1128–1136.

Mokhtar-Maamouri F. 1979. Étude en microscopie électronique de la spermiogenèse et du spermatozoïde de *Phyllobothrium gracile* Wedl, 1855 (Cestoda, Tetraphyllidea, Phyllobothriidae). *Zeitschrift für Parasitenkunde*, 59, 245–258.

Mokhtar-Maamouri F. 1982. Étude ultrastructurale de la spermiogenèse de *Acanthobothrium filicolle* var. *filicolle* Zschokke, 1888 (Cestoda, Tetraphyllidea, Onchobothriidae). *Annales de Parasitologie* (*Paris*), 57, 429–442.

Mokhtar-Maamouri F., Świderski Z. 1975. Étude en microscopie électronique de la spermatogénèse de deux cestodes *Acanthobothrium filicolle benedenii* Loennberg, 1889 et *Onchobothrium uncinatum* (Rud., 1819) (Tetraphyllidea, Onchobothriidae). *Zeitschrift für Parasitenkunde*, 47, 269–281.

Ndiaye P.I., Agostini S., Miquel J., Marchand B. 2003a. Ultrastructure of spermiogenesis and the spermatozoon in the genus *Joyeuxiella* Fuhrmann, 1935 (Cestoda, Cyclophyllidea, Dipylidiidae): comparative analysis of *J. echinorhynchoides* (Sonsino, 1889) and *J. pasqualei* (Diamare, 1893). *Parasitology Research*, 91, 175–186.

Ndiaye P.I., Miquel J., Marchand B. 2003b. Ultrastructure of spermiogenesis and spermatozoa of *Taenia parva* Baer, 1926 (Cestoda, Cyclophyllidea, Taeniidae), a parasite of the common genet (*Genetta genetta*). *Parasitology Research*, 89, 34–43.

Rausch R.L. 1994. Family Mesocestoididae Fuhrmann, 1907. In: *Keys to the cestode parasites of vertebrates* (Eds. L.F. Khalil, A. Jones and R.A. Bray). CAB International, Wallingford, 309–314.

Schmidt G.D. 1986. Handbook of tapeworm identification. CRC Press, Inc., Boca Raton, Florida, USA.

Sène A., Bâ C.T., Marchand B. 1997. Ultrastructure of spermiogenesis and the spermatozoon of *Nomimoscolex* sp. (Cestoda, Proteocephalidea) intestinal parasite of *Clarotes laticeps* (fish, teleost) in Senegal. *Journal of Submicroscopic Cytology and Pathology*, 29, 1–6.

Sène A., Bâ C.T., Marchand B. 1999. Ultrastructure of spermiogenesis of *Phyllobothrium lactuca* (Cestoda, Tetraphyllidea, Phyllobothriidae). *Folia Parasitologica*, 46, 191–198.

Shi D.Z., Liu D.S., Wang S.K., Craig P.S. 1994. The ultrastructure of *Echinococcus multilocularis*. *Chinese Journal of Parasitic Disease Control*, 7, 40–41.

Stoitsova S.R., Georgiev B.B., Dacheva R.B. 1995. Ultrastructure of spermiogenesis and the mature spermatozoon of *Tetrabothrius erostris* Loennberg, 1896 (Cestoda, Tetrabothriidae). *International Journal for Parasitology*, 25, 1427–1436.

Świderski Z. 1968. The fine structure of the spermatozoon of sheep tapeworm, *Moniezia expansa* (Rud., 1810) (Cyclophyllidea, Anoplocephalidae). *Zoologica Poloniae*, 18, 475–486.

Świderski Z. 1985. Spermiogenesis in the proteocephalid cestode *Proteocephalus longicollis*. *Proceedings of the Electron Microscopical Society of South Africa, 24th Annual Conference, Pietermaritzburg*, 181–182.

Świderski Z. 1986. Three types of spermiogenesis in cestodes. *Proceedings of the XIth International Congress of Electron Microscopy, Kyoto*, 2959–2960.

Świderski Z. 1994. Spermiogenesis in the trypanorhynchid cestode *Lacistorhynchus tenuis*. *Proceedings of the 13th International Congress on Electron Microscopy, Paris*, 691–692.

Świderski Z., Mackiewicz J.S. 2002. Ultrastructure of spermatogenesis and spermatozoa of the caryophyllidean cestode *Glaridacris catostomi* Cooper, 1920. *Acta Parasitologica*, 47, 83–104.

Świderski Z., Mokhtar-Maamouri F. 1980. Étude de la spermatogénèse de *Bothriocephalus clavibothrium* Ariola, 1899 (Cestoda: Pseudophyllidea). *Archives de l'Institut Pasteur de Tunis*, 57, 323–347.

Świderski Z., Salamatin R.V., Tkach V.V. 2000. Electron microscopical study of spermatozoa of the cestode *Dilepis undula* (Cyclophyllidea, Dilepididae). *Vestnik Zoologii*, 34, 3–7.

Tian X., Yuan L., Huo X., Han X., Li Y., Xu M., Lu M., Dai J., Dong L. 1998. Ultrastructural observations on the transformation of the spermatozoon in spermatogenesis of taeniid cestodes. *Chinese Journal of Parasitology and Parasitic Diseases*, 16, 269–273.

Wardle R.A., McLeod J.A., Radinovsky S. 1974. Advances in the zoology of tapeworms, 1950–1970. University of Minnesota Press, Minneapolis, USA.

Watson N.A., Rohde K. 1995. Sperm and spermiogenesis of the "Turbellaria" and implications for the phylogeny of the phylum Platyhelminthes. In: *Advances in spermatozoal phylogeny and taxonomy* (Eds. B.G.M. Jamieson, J. Ausió and J.-L. Justine). *Mémoires du Muséum National d'Histoire Naturelle, Paris*, 166, 37–54.

Willms K., Caro J.A., Robert L. 2003. Ultrastructure of spermatogonia and spermatocyte lobules in *Taenia solium* strobilae (Cestoda, Cyclophyllidea, Taeniidae) from golden hamsters. *Parasitology Research*, 90, 479–488.

Willms K., Robert L., Jiménez J.A., Everhart M., Kuhn R.E. 2004. Ultrastructure of spermiogenesis and the spermatozoon in *Taenia crassiceps* strobilae WFU strain (Cestoda, Cyclophyllidea, Taeniidae) from golden hamsters. *Parasitology Research*, 93, 262–267.

Yamaguti S. 1959. Systema helminthum. Vol II. The cestodes of vertebrates. Interscience Publishers, Inc., New York & London.